COBALT AND MANGANESE MESOPORPHYRINS IN COORDINATION WITH NITROGENOUS BASES

A dissertation submitted to the Board of University Studies, The Johns Hopkins University, in conformity with the requirement for the degree of Doctor of Philosophy

JOHN FULLER TAYLOR

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Foreword

Part I, which has to do chiefly with theoretical relations, is the joint contribution of Mr. Taylor, Mr. Davies, Mr. Vestling, and myself. Under this joint authorship it was published with the title *Metalloporphyrins. I. Coordination with nitrogenous bases. Theoretical relations* in The Journal of Biological Chemistry, **135,** 543 (1940).

Part II describes experimental results that were obtained by Mr. Taylor exclusively. It was published under his authorship with the title *Metalloporphyrins. II. Cobalt and manganese mesoporphyrins in coordination with nitrogenous bases* in The Journal of Biological Chemistry, **135,** 569 (1940).

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PART I. THEORETICAL

In this series of studies we shall contribute to the systematic description of the behavior of metalloporphyrins in their coordination with nitrogenous bases.

The first method of attack will be the potentiometric. The results will be supplemented by spectrophotometric measurements.

Conant and his students (1, 2) initiated the potentiometric exploration of systems containing heme and a base that coordinates with heme in both the oxidized and reduced states. Such studies have been extended by Barron and Hastings (3) and particularly by Barron (4). Also important contributions to our knowledge of these complex systems have come by way of spectrophotometry, notably at the hands of Hogness, Zscheile, Sidwell, and Barron (5). The assembly of facts thus made available clarifies relations discussed in the earlier, enlightening papers of Anson and Mirsky (6) and Mirsky and Anson (7).

We shall deal with several systems containing not only different bases but metalloporphyrins composed of different metals and different porphyrins; therefore we require an adaptable nomenclature. We shall report the evidence of considerable complexity; therefore we think it wise to outline with care both the presumptions and the restrictions of a theoretical approach. We shall

have to submit our data to various sorts of analysis. Since that of any one sort will be repeated, we shall conserve space by devoting the greater part of this paper to the development of propositions that can be submitted to experimental tests and that may be referred to later by numbers. Exploratory measurements, detailed in the dissertations of Taylor (8), Davies (9), and Vestling (10) are summarized in the following papers by these authors.

Nomenclature

Several of the terms commonly employed in this field stem from those which arose during the early study of the prosthetic group of mammalian blood pigments and are not etymologically good when applied to substances containing metals other than iron and porphyrins other than protoporphyrin IX. Some attempts at reform have left the nomenclature in more or less confusion. We had adopted a simple nomenclature when Drabkin (11) proposed essentially the same system.

A compound formed from a porphyrin and a metal ion is called a metalloporphyrin. The metal and its state may be designated by expressions such as *ferro*, *cobalti*, etc., as in *manganomesoporphyrin IX*. A compound formed from a metalloporphyrin and another substance is designated by prefixing the name of the coordinating substance to that of the metalloporphyrin, as in *nicotine ferriprotoporphyrin*. In running commentaries, where the specific substance is clearly understood, a compound such as cyanide ferrohematoporphyrin may be spoken of as a base metalloporphyrin, *base* here being used in a wide sense.

Our symbols will refer to nitrogenous bases as coordinating substances but we do not wish to imply that only nitrogenous bases are able to coordinate with metalloporphyrins.

It will be assumed that the preparative procedures and certain identifying tests, which confirm what others have described, suffice to identify our porphyrins. If there be occasion to define a porphyrin quite specifically, both the name and the number given in Fischer's (12) system will be used, as in the name *mesoporphyrin IX*.

Scope and General Definition of Problems

Experience in this laboratory has confirmed our guess that some of the general features of these systems will be revealed by

modifying properties pertaining to one or another class of the components. Accordingly, porphyrin, metal, and coordinating base have been changed independently.

What was not foreseen is that when components are modified each of the systems so far studied has some property that restricts severely the range of experimental study. Accordingly the data require some piecing together if one is to obtain a general formulation of the equilibria and a tentative picture of the coordination compounds that are involved. In attempting this we find it essential to have in view the types of reaction that are to be met and equations formulating those under special study. Indeed, both the older data and much of that gathered in our first experiments are inadequate, because there were lacking those formulations that could have indicated critical relations. Also, such criticism of our final conclusions as may be made should involve a scrutiny of the basic assumptions.

The phenomena observed to date show that the following types of reaction occur in systems containing a metalloporphyrin and a coordinating base. The list is restricted to reactions that are pertinent to the immediate subject. As occasion demands, a more detailed formulation will be constructed with the accumulating evidence and a few postulates, and this will serve to account for the ionic charges not mentioned in the following primitive formulations.

1. Reversible association between porphyrin and reduced metal ion.

Example—Ferrous ion + protoporphyrin $\rightleftharpoons$ ferroprotoporphyrin

It will be recognized that this type of reaction, studied by Fischer, Treibs, and Zeile (13), is involved in the preparation of porphyrins from metalloporphyrins and of metalloporphyrins from porphyrins and metal ions. Except under the special conditions which Vestling (10) describes in his study of preparative methods, it will be assumed that the equilibrium state of this reaction is far "to the right." This assumption seems to be generally accepted for the alkaline water, and water-alcohol solutions such as were used in our studies and accordingly we have not examined it with the care that is desired ultimately.

That metal ions combine with porphyrins containing no acidic group other than $\rangle$NH is the most convincing of the evidences

that the predominating species of the metalloporphyrins having carboxyl groups are not normal salts of the carboxylic acids. Other evidence has confirmed the belief that they are coordination compounds involving the pyrryl nitrogens. This will be so far the tacit assumption that our formulations will provide for only one sort of metalloporphyrin, which we have already called a "coordination compound."

2. Reversible oxidation and reduction of the metalloporphyrin, if the metal component be of appropriate type.

Example—Cobaltimesoporphyrin + electron $\rightleftharpoons$ cobaltomesoporphyrin

It seems unnecessary to review the evidence that there is a preferential response to oxidizing and reducing agents, and that when these agents are suitably apportioned the first response is determined by the state of the metal component and that groups characteristic of the porphyrin alone are but slightly affected, if at all. Since each of the metals that we have used is such that 1 equivalent is concerned in the reduction of the quantity of metalloporphyrin containing a gm. atom of metal, formulations will be simplified by retaining this specification.

3. Reversible dissociation of hydrions (or hydroxyl ions).

Since porphyrin, metalloporphyrin, coordinating base, and base metalloporphyrin may have distinctive, or, in some cases, common groups with different acidic dissociation constants, there are numerous theoretical possibilities for the integration of the free energies of acid ionization and of hydrion dilution with the free energy of the ideal oxidation-reduction process. Actually, of course, all may be integrated in what may be called a continuum (Clark (14)). We may simplify matters greatly by anticipating the evidence that under the limited conditions of the measurements the detected, dissociable hydrions (or hydroxyl ions) pertain to the coordination center rather than to groups characteristic of the porphyrin alone or of the coordinating base alone. Ionizations pertaining to the latter two sorts of groups have to be considered under other circumstances. For the most part the range of pH used in the experimental studies was such that the carboxyl groups of the porphyrin can be *assumed* to be ionized.

4. Reversible association between oxidized metalloporphyrin and a nitrogenous base (including cyanide ion) and between reduced metalloporphyrin and the same base.

Examples—

Manganomesoporphyrin + r pyridine $\rightleftarrows$ pyridine$_r$ manganomesoporphyrin

Manganimesoporphyrin + q pyridine $\rightleftarrows$ pyridine$_q$ manganimesoporphyrin

It is the difference between the dissociation constants of the oxidized and reduced base metalloporphyrins that makes possible the potentiometric evaluation of these constants. This is the unique feature of the following papers the mathematical introduction of which is given in this paper.

5. Formation of polymers of metalloporphyrin or of a base metalloporphyrin.

On the one hand there will be given evidence of the existence of dimers in certain instances. On the other hand this calls for equations that are very complex and some of them are not amenable to the approximations that serve as guides to the experimental study of the simpler cases. None the less, when occasion demands, the simpler cases can serve as guides to the treatment of the complex.

For the study of a system having even a part of the possible complexity suggested by this compilation, spectrophotometry has one apparent, but not always real, advantage over potentiometry. The latter requires the presence of the oxidized and the reduced components, greatly increasing the number of components; yet electrode measurements provide certain basic data by means of which the free energy changes may be aligned with those of other oxidation-reduction systems. We need not foreshow the importance of this. What may be emphasized now is that each method has distinct limitations.

In order that the part contributed by each reaction to the free energy change of the continuum may be expressed in a manner that helps our understanding of these complex systems, we should have equations relating the experimentally determinable quantities.

A general equation would be unwieldy were it to describe the electrode potential as a function of the activities of all the components that are implied in this list of reactions. Therefore we had best break down the continuum of actually integrated processes and describe the parts by a plexus of equations. Happily, the control of hydrogen ion activities by means of buffer solutions permits us to eliminate consideration of the part played by hydrogen ion activity while developing other features and to deal

with the "pH effect" separately. Type equations for acid-base equilibria need no repetition.

Equations for Association

There remain for particular consideration the equilibria involving the associations between a nitrogenous base and a metalloporphyrin in the reduced and oxidized states. These are unique. Equations for a type case were first developed by Clark, Taylor, Davies, and Lewis (15) from the elementary equation of Conant and Tongberg (2). Only so much of the development will be given here as is necessary to outline the subject and to provide those typical equations that guide experiment. More detail will be found in the dissertations of Taylor (8), Davies (9), and Vestling (10).

Assume a metalloporphyrin that is subjected to oxidation-reduction in the sense

$$\mathbf{O}_n + ne \rightleftharpoons \mathrm{p}\mathbf{R}_m$$

where $\mathbf{O}_n$ represents the oxidant in the fixed state of aggregation designated by n, which is also the number of electrons, e, required for the reduction, and $\mathbf{R}_m$ represents the reductant in the fixed state of aggregation designated by m. If n be greater than 1, we shall now assume no intermediate state of reduction, although it will be important to reconsider this limitation should evidence make it necessary.

$$\mathrm{p}m = n \tag{1}$$

Let both species be under experimental conditions developing no fact that demands consideration of their acid ionizations or ionic charges, as would be necessary to "balance" the "equation" on one side of which appear n electrons. The electrode equation involving accepted conventions[1] is Equation 2.

$$E_h = E_0 + \frac{RT}{nF} \ln \frac{(\mathbf{O}_n)}{(\mathbf{R}_m)^{\mathrm{p}}} \tag{2}$$

Let a nitrogenous base, B, be added to the solution and there coordinate independently with the oxidized and the reduced

[1] See Clark (16).

metalloporphyrin to form the compounds defined by the following relations.

$$\mathbf{O}_n + q\mathrm{B} \rightleftharpoons \mathbf{O}_n\mathrm{B}_q$$

$$\mathbf{R}_m + r\mathrm{B} \rightleftharpoons \mathbf{R}_m\mathrm{B}_r$$

With brackets to indicate molar concentrations, parentheses to indicate activities, and γ to indicate activity coefficients we may state the equilibrium equations in two forms:

$$\frac{(\mathbf{O}_n)(\mathrm{B})^q}{(\mathbf{O}_n\mathrm{B}_q)} = K_O \qquad (3, a)$$

$$\frac{[\mathbf{O}_n][\mathrm{B}]^q}{[\mathbf{O}_n\mathrm{B}_q]} = \frac{\gamma_{O_n\mathrm{B}_q}}{\lambda_{O_n}\gamma_{\mathrm{B}^q}} K_O = K'_O \qquad (3, b)$$

$$\frac{(\mathbf{R}_m)(\mathrm{B})^r}{(\mathbf{R}_m\mathrm{B}_r)} = K_R \qquad (4, a)$$

$$\frac{[\mathbf{R}_m][\mathrm{B}]^r}{[\mathbf{R}_m\mathrm{B}_r]} = \frac{\gamma_{R_m\mathrm{B}_r}}{\gamma_{R_m}\gamma_{\mathrm{B}^r}} K_R = K'_R \qquad (4, b)$$

An important assumption is that there is no change in the state of aggregation when metalloporphyrin is converted to base metalloporphyrin.

The assumptions noted will deserve strict attention when the precision of measurement and control of all factors reach artistic adjustment to theoretical requirements. Some will receive further, qualitative treatment later.

Define the following sums

$$S_o = n[\mathbf{O}_n] + n[\mathbf{O}_n\mathrm{B}_q] \qquad (5)$$

$$S_r = m[\mathbf{R}_m] + m[\mathbf{R}_m\mathrm{B}_r] \qquad (6)$$

With the equations at hand derive Equation 7, a.

$$E_h = E_0 + \frac{RT}{nF}\ln\frac{S_o}{S_r{}^p} + \frac{RT}{nF}\ln\frac{m^p}{n} + \frac{RT}{nF}\ln\frac{K'_O}{(K'_R)^p}$$

$$+ \frac{RT}{nF}\ln\frac{(K'_R + [\mathrm{B}]^r)^p}{K'_O + [\mathrm{B}]^q} + \frac{RT}{nF}\ln\frac{\gamma_{O_n}}{\gamma_{R_m{}^p}} \qquad (7, a)$$

If the conditions are such as to permit no serious change in the values of the activity coefficients occurring in Equations 3, b

and 4, *b*, we may proceed as if the last term in Equation 7, *a* were constant and K'_O and K'_R were constant. To simplify the typography we shall omit primes of K'_O and K'_R hereafter.

Proposition I—When all conditions are constant except the ratio of S_o to $(S_r)^p$, symmetry of the titration curve is presumptive evidence that p = 1. See also Proposition XII, *b*.

Proposition II—If p = 1, the slope of the titration curve gives the value of *n*.

Practically this is best determined by Proposition XVI. If p = 1, $m = n$. If it be found that $p = m = n = 1$ when sufficient base is present to convert the oxidized and reduced metalloporphyrins practically completely to the respective base metalloporphyrins, it will not necessarily hold that $p = m = n = 1$ for the metalloporphyrin system itself.

We shall continue with the assumption that $p = n = m = 1$ and equal activity coefficients. These assumptions reduce Equation 7, *a* to 7, *b*.

$$E_h = E_0 + 0.0601 \log \frac{S_o}{S_r} + 0.0601 \log \frac{K_O}{K_R} + 0.0601 \log \frac{K_R + [B]^r}{K_O + [B]^q} \quad (7, b)$$

There is included the condition that the temperature is 30°, since the consequences of the numerical coefficient are to be shown graphically.

If [B] be held practically constant, we have Equation 8, to be

$$E_h = E_b + 0.0601 \log \frac{S_o}{S_r} \quad (8)$$

tested by Propositions I and II. Here E_b indicates the potential that would be observed at 50 per cent reduction of the system at constant concentration of free base, which in most instances will obtain practically at constant concentration of total base. If no base be present, $E_b = E'_0$.

Proposition III—If the potential increase with addition of coordinating substance, all other conditions remaining constant, $K_O > K_R$. If the potential change is in the opposite direction, $K_R > K_O$.

We shall confine illustration to the first case.

Proposition IV—If on addition of base, all other conditions remaining constant, the potential approaches a limiting potential, E_2 (see Equation 11), it follows that $q = r$.

The difference $E_2 - E_0$ is determined by the ratio, K_O/K_R, as shown by Equation 11 and as illustrated in Fig. 1. The course of the association curve between E_0 and E_2 is determined by the absolute values of K_O and K_R, and by the concentration of total metalloporphyrin, S.

Particular emphasis may be given to one conclusion that flows from a consideration of these systems and that is implicit in the equations. This is that the change of potential, accompanying the addition of a coordinating base to a fixed mixture of the reduced and oxidized species of the metalloporphyrin, is a measure of the *ratio* of the two dissociation constants. This difference betokens a difference in the free energies of association and where this is large it may need to be considered in the ultimate analysis of the part that complexes of the metalloporphyrins play in catalysis. Furthermore, the fact that other catalysts found in living cells depend upon the association between dissociable prosthetic groups and specific proteins suggests that base metalloporphyrin systems may serve in some degree as models of important general relations. Barron (4) has already pointed to the fact that the potentials of systems containing the same metalloporphyrin can vary considerably with the nature of the base. These specific effects of coordinating bases are referable to the specific values of K_O and K_R. Barron and Hastings (3) and Barron, DeMeio, and Klemperer (17) have applied the shift of potential to the modification of catalytic action.

It is desired to develop Equation 7, b to include the concentration of total coordinating base, S_b, which is directly determinable. The concentration of total base is defined by Equation 9. Other definitions and specifications convenient to the development follow.

$$S_b = [\mathrm{B}] + q[\mathrm{OB}_q] + r[\mathrm{RB}_r] \tag{9}$$

$$S = S_o + S_r \tag{10}$$

$$E_2 = E_0 + 0.0601 \log \frac{K_O}{K_R} \tag{11}$$

$$x = \frac{[O]}{[R]} = \log^{-1} \frac{(E_h - E_0)}{0.0601} \tag{12}$$

$$y = \frac{[OB_q]}{[RB_r]} = \log^{-1} \frac{(E_h - E_2)}{0.0601} \tag{13}$$

$$\alpha = \frac{S_o}{S} \tag{14}$$

$$\beta = \frac{[OB_q] + [RB_r]}{S} \tag{15}$$

With these relations Equation 16 may be derived.

$$E_h = E_0 + 0.0601 \log \frac{S_o}{S_r} + 0.0601 \log \frac{K_O}{K_R} + 0.0601 \log \frac{K_R + (S_b - ZS)^r}{K_O + (S_b - ZS)^q} \tag{16}$$

where, for the restriction that $q = r$

$$Z = \frac{r(y+1)}{(x-y)} [x(1-\alpha) - \alpha] \tag{17}$$

and for the further restrictions that $\alpha = 0.5$ and $r = 2$

$$Z = \frac{(y+1)(x-1)}{(x-y)} \tag{18}$$

For the restriction that $\alpha = 0.5$ but no limitation on the relation of q to r

$$Z = \frac{(qy+r)(x-1)}{(x-y)} \tag{19}$$

Proceed, for the moment, with Equations 16 and 18.

Proposition V—Certain first order, graphical approximations may give useful information, as detailed below.

In the case selected, unique points of the association curve are (a) where

$$[B]^2 = K_R \text{ or } 2 \log [B] = \log K_R \tag{20}$$

There $Z = 0.5$, $E_h - E_0 \fallingdotseq 0.0181$, $S_b \fallingdotseq K_R - 0.5S$. The last two relations and the known values of S_b and S make it possible to judge when $S_b \fallingdotseq [B]$. This approximation is least valid at the low parts of the association curve, but, especially when the value of S is low, it will be found frequently that it may be used profit-

ably if not extended below this first unique point. Then S_b may replace [B] in Equation 7, *b* and modified Equation 7, *b* may be used in place of the unwieldy Equation 16; (*b*) the center point of inflection where

$$[B]^4 = K_O K_R \tag{21}$$

There $Z = 1$, $[B] = (S_b - S)$, $\beta = 0.5$, $[RB_r] = [O]$, $[R] = [OB_q]$, and $E_h = (E_0 + E_2)/2$; (*c*) where

$$[B]^2 = K_O \text{ or } 2 \log [B] = \log K_O \tag{22}$$

There $Z = 1.5$ and $E_2 - E_h = 0.0181$.

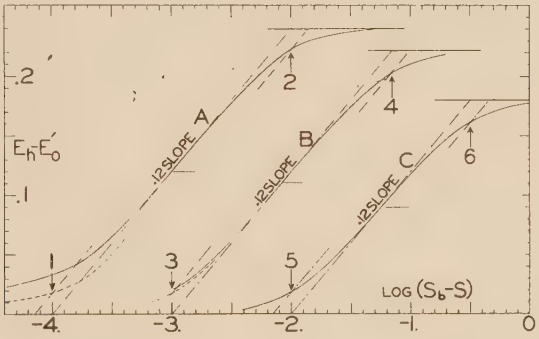

FIG. 1. The solid lines represent the theoretical relation of $E_h - E'_0$ to log $(S_b - S)$ when $q = r = 2$, $0.5S = S_r = S_o = 1 \times 10^{-4}$ M; the broken lines, the same with log $(S_b - 0.5S)$ as abscissa. Curve A, $K_R = 1 \times 10^{-8}$, 0.5 log $K_R = -4.00$ (see Point 1); $K_O = 1 \times 10^{-4}$, 0.5 log $K_O = -2.00$ (see Point 2); $E_2 - E_0 = 0.2400$ (0.06 coefficient). Curve B, $K_R = 1 \times 10^{-6}$, 0.5 log $K_R = -3.00$ (see Point 3); $K_O = 5 \times 10^{-3}$, 0.5 log $K_O = -1.15$ (see Point 4); $E_2 - E_0 = 0.2219$ (0.06 coefficient). Curve C, $K_R = 1 \times 10^{-4}$, 0.5 log $K_R = -2.00$ (see Point 5); $K_O = 1 \times 10^{-1}$, 0.5 log $K_O = -0.50$ (see Point 6); $E_2 - E_0 = 0.1800$ (0.06 coefficient).

For the case under consideration it may be shown that, when $K_O > S_b{}^r > K_R$ at the center point, the slope at the mid-point approaches the uniquely simple, algebraic form (Equation 23):

$$\frac{dE}{d \log(S_b - S)} \doteq (0.0601)r \tag{23}$$

Fig. 1 illustrates a method of graphical analysis based upon the use of these approximations and unique points. Since Z is

unity at the center point, log $(S_b - S)$ is made the abscissa in order that the important orienting diagonal, of slope 0.12, may coincide with the center point. When the ratio of K_O to K_R is as large as any of the values indicated, the diagonal is very close to the tangent through the mid-point and accordingly can be well placed. This diagonal will intersect the line of E'_0 where

$$0.5 \log K_R = \log [B] \tag{24}$$

Now if $[B] \fallingdotseq (S_b - S)$, as it does in Curve C, this point of intersection will be 0.0181 volt below the corresponding point of the association curve, drawn with log $(S_b - S)$ as abscissa. Curves B and A show progressively greater departure from this relation. Yet at the unique point in question $Z = 0.5$, so that, if a supplementary curve be plotted with log $(S_b - 0.5S)$ as abscissa, the unique point falls on the supplementary curve very close to 0.0181 volt above the aforementioned intersection. We shall show that it is not always possible to report a reliable value for E'_0. When deprived of this base-line, one may use the obvious, alternative method shown in Fig. 1; namely, to intersect the appropriate curve with a line parallel to the orienting diagonal and 0.0181 volt above. For the curves shown and on the scale used in Fig. 1 it would be difficult to discern any difference along the upper part of the curves whether the abscissa were made log $(S_b - S)$, log S_b, or the log $(S_b - 1.5S)$ which is demanded for the location of the unique point where

$$0.5 \log K_O = \log [B] \tag{25}$$

If necessary a drawing of larger scale may be used and the principle described for the finding of K_R applied to the estimation of K_O. Fig. 1 illustrates the elements.

Once the orders of magnitude of K_O and K_R, as well as of S, are known, one may estimate the legitimacy of each approximation. In extreme cases it may be necessary to resort to a series of progressively closer approximations but, especially when S is small and the constants large, it suffices to plot with log S_b. The latter is preferable for final description.

It should not go unnoted that previous authors have plotted with S_b as abscissa. The resulting graph obscures those features that are useful in the estimation of K_R and K_O and it may leave

the impression that the potentials at high concentration of base have reached a limit when the evidence thereof is quite inadequate.

Proposition VI—If at and near the center of an association curve K_O is much greater than $[B]^q$ and K_R is much less than $[B]^r$, the value of r will be determined by the maximal slope of the association curve, which is given by Equation 23.

The actual slope need only approximate an integral multiple of 0.0601, since r must be a whole number. The departure will be the greater the smaller the ratio of K_O to K_R. If q be less than r, the effect of combination between base and oxidant will extend over a larger range and may affect the slope of the midsection.

Comment on Stepwise Association—If association be stepwise, a term such as $K_O + [B]^2$ in Equation 7, b must be replaced by

$$K_{O_1}K_{O_2} + K_{O_1}[B] + [B]^2 \qquad (26)$$

or by a more extended term if the number of steps be greater than two. On statistical grounds stepwise association is more probable than concurrent addition of 2 or more molecules of base and under certain distributions of the energy changes this may be reflected in the equilibrium states. Therefore the theorist will be tempted to provide for this. On the other hand the experimentalist will recognize that the provision of several constants in terms of this kind is not unlike the provision of several constants in those infinite series that are used for the development of empirical equations and becomes devoid of physical significance if his method be not delicate enough to detect the individual values. But be it noted that, as [B] varies, the value of the sum in Equation 26 runs from that of the first term to that of the last. Accordingly, for the purpose of defining the gross contour of an association curve, Equation 26 may be reduced to the corresponding term in Equation 7, b.

The further the departure between the values of the first and second dissociation constants, the more will be the weight of a $K_1[B]$ term, so that experimental identification may become possible. When it is, the equations may be enlarged appropriately.

A Note on Polymers—The potentiometric method can reveal

the presence of polymers. A particular case was described by Stiehler and Clark (18). Barron (4) and Davies (9) show that the components of certain metalloporphyrin systems are dimers. The equations here given are predicated on the assumption that there is no *change* in state of aggregation and where the symbols that have been used imply monomeric components the equations need only slight modification to represent the case in which all metalloporphyrins are dimeric. The situation is entirely different when there is a *change* in state of aggregation accompanying association with a base. Because of the much greater complexity

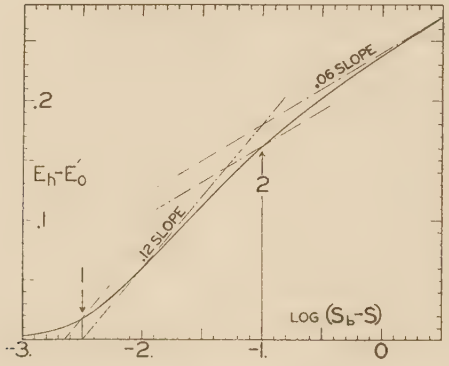

FIG. 2. Theoretical curve. Case of $q = 1$, $r = 2$, $K_R = 1 \times 10^{-5}$, $K_O = 1 \times 10^{-1}$. Point 1, 0.5 log K_R = log ($S_b - S$); Point 2, log K_O = log ($S_b - S$).

of such equations we think it wise to postpone their treatment until necessity demands it.

Cases in Which $q < r$ —For such cases Propositions I, II, III, and VI will hold, while IV and V need modification. Fig. 2 illustrates a theoretical case and a method of graphical analysis so similar to that already described as to require no further comment. Fig. 2 will also serve to illustrate some of the propositions that follow.

Proposition VII—If $q < r$, the maintenance of the slope r (0.0601) at high values of [B] should indicate that K_o is infinite, and $q = 0$ (no association of oxidized metalloporphyrin and base).

Proposition VIII—A decline of the slope on further addition of base indicates that K_o is finite and $q > 0$.

On still further addition of base the term 0.0601 log $(K_R + [B]^r)/(K_o + [B]^q)$ approaches 0.0601 log $[B]^r/[B]^q$ or 0.0601 log $(S_b)^{r-q}$ (see Fig. 2). Whence:

Proposition IX—If, at very high concentration of coordinating base, the slope of the association curve approaches a constant value, the slope is given by Equation 27 from which is determined the value of $r - q$.

$$\frac{\Delta E_h}{\Delta \log S_b} = 0.0601(r - q) \qquad (27)$$

Participation of Hydrions—To avoid unnecessary complexity we have followed the policy of breaking down the continuum of actually integrated processes and up to this point have left out of consideration the participation of hydrogen (or hydroxyl) ions. That this is a practical policy flows from the established fact that, if a set of measurements be made in buffer solutions at constant pH, the components of the buffer system will absorb or supply the hydrogen ions liberated or taken up in the reactions hitherto considered and no experimental evidence of their participation will appear, provided, of course, that the buffer system be in great excess. By the same token, a shift in the pH value of the buffer system will alter the ease with which hydrogen ions are made available for the completion of the reaction and the effect will appear in the magnitude of the over-all free energy change, the intensity factor of which is measured by the electrode method.

Since these principles have been developed in previous studies (19), it seems unnecessary to embody them in equations now being used to outline general features, although it will be essential to do so in dealing with specific, actual cases. But there is one aspect that should be given particular emphasis lest the policy now being followed become grievously deceptive.

Prior to potentiometric studies by Conant (1, 2) and Barron (4) it was largely a matter of artistry to assign to ferriprotoporphyrin in alkaline solution a hydroxyl ion, or a species formula that we shall represent by $O\overline{OH}$. Now that potentiometric measurements have shown hydrogen ions or hydroxyl ions to be involved in the equilibria obtaining among some of the systems, we must contemplate the possibility that there is a competition between hydroxyl ions and coordinating base molecules for posi-

tion at the coordination center. To take care of this in the simplest of several possible formulations let us postulate that in the coordination between base and oxidized metalloporphyrin the base coordinates only with the species O, leaving applicable Equation 3, *b* but that, in addition, there obtains the equilibrium represented in Equation 3, *c*.

$$\frac{[\overline{OOH}][H^+]}{[O]} = k[H_2O] = K_h \qquad (3, c)$$

By following the usual procedure with the inclusion of Equation 3, *c* one arrives at an "electrode equation" comparable with Equation 7, *b* and differing therefrom only in the last term. This term when written in a form suited to the immediate discussion is

$$0.0601 \log \frac{K_R + [B]^r}{\frac{K_O}{[H^+]}([H^+] + K_h) + [B]^q} \qquad (28)$$

The point to be emphasized is that when association experiments are run at *constant* hydrogen ion concentration the constant called K_O in the foregoing discussion should be in reality $(K_O/[H^+])([H^+] + K_h)$. In other words what has been called the dissociation constant of the base metalloporphyrin in oxidized form is an "apparent constant" the value of which is a function of $[H^+]$.

The general significance of this warning is not dependent upon the particular postulate used above—a postulate which we shall show is too simple.

Proposition X—Previous theoretical and experimental studies (19, 20) have shown that, when the relation between E_h and pH, other conditions being constant, is

$$\frac{-\Delta E_h}{\Delta \text{ pH}} = 0.0601 \text{ (at } 30°) \qquad (29)$$

the reductant must possess 1 more hydrogen ion (or 1 less hydroxyl ion) than the oxidant for each electron necessary to convert oxidant to reductant.

Evidence will be presented in the experimental papers that the pH effects have to do with the coordination shell so that we may now say that the above relation will demonstrate the reduced

coordination center to possess 1 more hydrogen ion or 1 less hydroxyl ion per metal atom.

Proposition XI—On increase of pH, if the *reductant* lose a equivalents of protons per mole, the value of $-dE_h/d$ pH will decrease by $a(0.0601)/n$ (at 30°); while if the *oxidant* lose a equivalents of protons per mole the value of $-dE_h/d$ pH will increase by $a(0.0601)/n$. Loss of a proton is equivalent to gain of hydroxyl ion. See references (19) and (20) for details on this proposition.

An example will be shown of a change from $-\Delta E_h/\Delta$ pH = 0.0601, for the higher values of pH, to $-\Delta E_h/\Delta$ pH = 0.0 for the lower values of pH. Whatever the structural interpretation may be, this experimental fact demands the *equivalent* of the loss of hydroxyl ion from the oxidant at the lower pH values. Also the mid-point of the transition between the two slopes gives the so called pK value.

Comment on Activities—There remain for consideration troublesome questions arising from changes in activity coefficients that have been neglected up to this point. A rigid treatment of this subject, involving so complicated a set of components, would require a length out of proportion to the scope of the experiments to be reported. Therefore, we may reduce our considerations to the following brief summary of our policy regarding the least certain of the several experimental aspects of this subject.

Simultaneous control of all factors but the one under immediate investigation is not rigidly attained in actual experiments. The greater uncertainties lie in the interpretation of data purporting to yield "pH" values and in corrections for those changes of pH and other factors that attend the modification of the medium. To provide a basis for the discussion of the first it seems advisable to outline here the experimental procedure.

pH Control—In many cases the concentration of added base, such as pyridine or nicotine, is so large as to make impracticable the use of the pH numbers of the solutions containing only the buffer salts. Furthermore, such substances interfere with the proper operation of the hydrogen electrode in the solutions used. Accordingly the glass electrode was employed. The type introduced by MacInnes and Belcher (21) was operated in a shielded, constant temperature, air bath. A Compton quadrant electrome-

ter was used for null point instrument in conjunction with a Leeds and Northrup type K potentiometer. A very close approach to the theoretical change of potential with pH was established with acid and neutral buffers that had been measured with the hydrogen electrode. The glass membrane had to be used with alkaline solutions with which there are well known difficulties. The use of potassium in place of sodium in the buffers materially reduced drifts and deviations. Since the membrane of Corning No. 015 glass behaved normally with mildly acidic buffers, if previous contact with alkaline buffers had not been prolonged, it was assumed that its initial response in alkaline solution was the more reliable. Accordingly rapid measurements were made of the tested solution and of buffer solutions of nearly the same pH numbers, as determined by the hydrogen electrode, and interpolations were made. By this procedure there were found orderly relations between the potentials of the cell with glass membrane and the amounts of alkali added to any one of the various buffer solutions and also orderly relations between the potentials and the amounts of pyridine or alcohol added to aqueous buffers.

If we accept a potential so measured as indicative of a pH number, that number appears to be referred to the standard for aqueous solutions and as if measured with the hydrogen electrode, since the latter remained the means of standardizing the compared solutions. Actually the higher concentrations of pyridine, or nicotine, which in some cases went as high as 3 M, may seriously impair the ordinary interpretation of "pH" and of the standard methods of its computation. Nevertheless, if any one set of data be restricted to solutions having grossly the same composition and if it then be found, on making a "pH" change the major variable, that there is established a close approach to the pH effect predicted by the simplified equations, the following conclusion is drawn. While strict standardization of pH numbers is precluded, the experimental satisfaction of the predicted pH effect in terms of *relative* pH changes establishes the *nature* of a part of the equilibrium. This, rather than precisely referred values, we consider to be of first order importance.

The greater uncertainties are in the experiments purporting to show the course of association. Here occur the largest of the uncontrolled changes of the medium. What we have been forced

to assume are the following. (*a*) The shift of pH accompanying the addition of neutral pyridine, nicotine, etc., is given by glass electrode measurements and can be applied by use of the established pH effect so as to bring all points on the association curve to the basis of constant "pH" on the arbitrary scale. (*b*) The shift of the activity coefficients is *assumed* to be negligible while coordinating base is increased. Here we may retain the same order of magnitude of the ionic strength but the dielectric constant will change.

APPLICATIONS OF SPECTROPHOTOMETRY[2]

Granting that obvious prerequisites for spectrophotometry are met and that *only two* species containing metalloporphyrin absorb at a suitably chosen wave-length, we may use the following relations.

Assume the process

$$aM_m + bA \rightleftharpoons cM_nA_e \tag{30}$$

where A represents either (*a*) base coordinating with M_m at constant pH to form M_nA_e or (*b*) hydrion adding to M_m to form M_nA_e at constant concentration of base. In case (*b*), M may be either metalloporphyrin or base metalloporphyrin. Relation 30 is stated in this general form for economy of space and to avoid needless repetition for the several specific cases. The restriction to two absorbing species places a severe limitation upon the following relations. Relief from this restriction will be noted in a special, experimental case.

Implied in Relation 30 are that:

$$am = cn \tag{31}$$

$$b = ce \tag{32}$$

$$\frac{[M_m]^a[A]^b}{[M_nA_e]^c} = K \tag{33}$$

Define

$$S = m[M_m] + n[M_nA_e] \tag{34}$$

$$\epsilon' = -\log T \tag{35}$$

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[2] See also Hogness *et al.* (5).

where T is the transmittance for any mixture of absorbing species at a fixed value of S and given length of absorbing column.[3]

$$\epsilon'_1 = -\log T_{Mm} \tag{36}$$

where T_{Mm} is the limiting value of T where $S = m[M_m]$.

$$\epsilon'_2 = -\log T_{MnAe} \tag{37}$$

where T_{MnAe} is the limiting value of T when $S = n[M_nA_e]$. By application of the rule of the additive property of log transmittances one obtains

$$\frac{[M_m]^a}{[M_nA_e]^c} = \frac{K}{[A]^b} = S^{a-c}n^cm^{-a}(\epsilon'_1 - \epsilon'_2)^{c-a}\frac{(\epsilon'_2 - \epsilon')^a}{(\epsilon' - \epsilon'_1)^c} \tag{38}$$

Proposition XII—(*a*) Symmetry of the curve relating ϵ' to log $[A]$ is presumptive evidence that $a = c$, whence $m = n$.

(*b*) Absence of a "dilution effect" involving S^{a-c} is further evidence. When $a = c$ and $m = n$, Equation 38 reduces to Equation 41 with appropriate interpretations of b and K.

If S is constant, the tangent to the curve at the mid-point, where $\epsilon' - \epsilon'_2 = \epsilon'_1 - \epsilon'$, will be given by

$$\frac{d\log[A]}{d\epsilon'} = \frac{-0.8686}{(\epsilon'_1 - \epsilon'_2)}\left(\frac{a+c}{b}\right) \tag{39}$$

Practically the use of this tangent may not be very certain, but to illustrate uses, consider the following possible cases.

Case 1. $M + 2A \rightleftharpoons MA_2$; $a = 1$, $b = 2$, $c = 1$
" 2. $2M + 3A \rightleftharpoons M_2A_3$; " = 2, " = 3, " = 1
" 3. $M_2 + 2A \rightleftharpoons M_2A_2$; " = 1, " = 2, " = 1

All of these give the same value of $(a + c)/b$. Yet Cases 2 and 3 would require 2 equivalents for the oxidation-reduction of the *base* metalloporphyrin. Therefore, if a symmetrical, potentiometric curve involving 1 equivalent were to be found, Case 1 could be presumed to apply and the slope now under discussion would indicate that $b = 2$. An additional and better test for

[3] The symbol ϵ' is introduced for economy of space in the more complicated equations. With apologies to the reader we ask that he carefully distinguish ϵ' from ϵ (see Table I).

the value of b is discussed in the following section. Be it noted that the spectrophotometric test alone would not distinguish Case 1 from Case 3.

Proposition XIII—If $a = c$ the center of the symmetrical curve relating ϵ' to log $[A]$ is where $\epsilon'_2 - \epsilon' = \epsilon' - \epsilon'_1$ and at this point

$$\log K = b \log [A] \tag{40}$$

RECTIFICATION OF CURVES

The general method of Reed and Berkson (22) is used.

Rectification of Spectrophotometric Curves. Relation of $-$ *Log* T (*or* ϵ') *to Log A*—This is limited to the following modification of Equation 38.

$$\frac{[M_m]}{[M_m A_b]} = \frac{K}{[A]^b} = \frac{\epsilon'_2 - \epsilon'}{\epsilon' - \epsilon'_1} \tag{41}$$

Choose an orienting pair of values called ϵ'_0 and log $[A]_0$ and define

$$b \log [A] - b \log [A]_0 = p \tag{42}$$

$$\log K - b \log [A]_0 = -\log G \tag{43}$$

where $p = 0$, when $\epsilon' = \epsilon'_0$. Derive

$$G\, 10^p = \frac{\epsilon' - \epsilon'_1}{\epsilon'_2 - \epsilon'} \tag{44}$$

$$G = \frac{\epsilon'_0 - \epsilon'_1}{\epsilon'_2 - \epsilon'_0} \tag{45}$$

Whence

$$\epsilon' = \epsilon'_1 + (G + 1) \frac{(\epsilon'_0 - \epsilon')10^p}{1 - 10^p} \tag{46}$$

Proposition XIV—If the assumed value of b fits the actual case, a chart of ϵ' against $((\epsilon'_0 - \epsilon')10^p)/(1 - 10^p)$ should give a straight line the properties of which determine K, ϵ'_1, and ϵ'_2.

The slope is $(G + 1)$, whence K is found by Equation 43. The intersection with the ϵ' axis gives ϵ'_1. Thence ϵ'_2 is found by Equation 45. If desired, the equation can be recast to yield ϵ'_2 by intersection.

Rectification of Potentiometric Association Curves Relating E_h to Log S_b—Let Equation 7, *b* be solved for $[B]^q$ under the condition that $q = r$. Assume $[B] \fallingdotseq S_b$. Define

$$\log^{-1} \frac{E_h}{0.0601} = u \tag{47}$$

$$\log^{-1} \frac{E_2}{0.0601} = J \tag{48}$$

$$q \log S_b - q \log S_{bo} = p \tag{49}$$

$$-q \log S_{bo} = \log C \tag{50}$$

The chosen pair of orienting data are u_o and S_{bo} where $p = 0$. Derive

$$u = J + (CK_O + 1) \frac{u_o - u}{10^p - 1} \tag{51}$$

Proposition XV—When previous knowledge justifies the use of the approximation carried into Equation 51, the slope of the straight line relating u to $(u_o - u)/(10^p - 1)$ is $(CK_O + 1)$ which yields the value of K_O by use of Equation 50 and the intersection with the u axis yields E_2 by Equation 48.

Similar relations leading to the evaluation of K_R and E'_0 could be obtained by use of $u' = \log^{-1} (-E_h/0.0601)$ and $J' = \log^{-1} (-E_0/0.0601)$.

The use of this method of rectification is tricky for the following reasons.

If the orienting values of u_o and $\log S_{bo}$ be taken from near the center of the association curve where the effects of K_O and K_R are small, the slope of the rectified line approaches unity and is of no value in the determination of either constant. The approximation carried into the equation, namely $S_b \fallingdotseq [B]$, is least valid at the lower part of the association curve. Therefore we hesitate to use this method for evaluating K_R. The following simpler rectification may be applicable in rare cases. Assume $q = r$, $S_o = S_r$, $S_b = [B]$, $K_O >> [B]^q$. Whence $E_h = E'_0 + 0.0601 \log (K_R + S_b{}^r)/K_R$. Define $u = \log^{-1} (E_h/0.0601)$ and $E_0 = 0.0601 \log W$. Whence $u = W + (W/K_R) S_b{}^r$. Thus a plot of u against $S_b{}^r$ should approximate a straight line the proper-

ties of which allow estimate of K_R but only a crude estimate of E'_0.

Rectification of Titration Curves

Reed and Berkson's method as applied to this case has been discussed by Clark and Perkins (23). We shall use the symbols defined by the latter authors as follows:

$y \equiv$ number of ml. of specified reducing agent required to reach the potential E_h

$d \equiv$ number of ml. of the reducing agent added before the main system is attacked

$A \equiv$ number of ml. of the reducing agent required to carry the main component from 0 to 100 per cent reduction

E_n and y_n are chosen orienting values of E_h and y respectively

$$P = \frac{E_h + E_n}{1/n(0.0601)} \tag{52}$$

$$E'_0 + E_n = -\frac{0.0601}{n} \log C \tag{53}$$

Whence

$$y = (C + 1)\frac{y_n + y10^P}{1 - 10^P} - dC \tag{54}$$

Proposition XVI—The properties of a titration curve are determined by the properties of a straight line the coordinates of which are y and $(y_n + y10^P)/(1 - 10^P)$ according to Equation 54.

The mid-point potential, E'_0, is determined by the slope $(C + 1)$ and thence all other characteristics of the titration curve are evaluated as described by Clark and Perkins.

The method is predicated on the applicability of Equation 8 and in particular upon an integral value of n. It is important to note the following. It is possible that the method may yield such numerical values of the constants that a theoretical, sigmoid curve, constructed with these values, may be shifted to a position of best correspondence with an actual titration curve that is slightly asymmetric. To be sure, only a slight asymmetry would go undetected; nevertheless there remains danger of overlooking something. For the present we may say that we know

of no better criterion of the validity of deduced values to be reported unless it be so extreme a precision of measurement and so rigid a control of variables as to make the mathematical analysis by the method of Reed and Berkson superfluous. All titration data are analyzed by this method.

SUMMARY

A nomenclature for metalloporphyrins and their coordination compounds is proposed.

The reactions that may take place in systems containing metalloporphyrins and coordinating bases are typed to define the restrictions of the immediate study.

There are developed theoretical equations relating the electrode potential, the concentration of total metalloporphyrin, the total base that coordinates independently with reduced and oxidized metalloporphyrin, and the ratio of concentrations of oxidized and reduced metalloporphyrin.

A brief review is given of the principles governing the study of the part played by the acid-base equilibria and of experimental difficulties in the application of these principles to the cases at hand.

Approximate forms of the equations are developed to show graphical methods of determining the constants that are descriptive of the dissociation of oxidized and reduced coordination complexes and to show methods of determining the numbers of molecules of coordinating substance that associate on the one hand with reduced metalloporphyrin and on the other hand with oxidized metalloporphyrin.

Equations are given for the use of spectrophotometric data in determining the various constants that define the equilibria of the systems.

Equations are given for rectification of curves.

Sixteen propositions and corollaries thereto are offered as subject to the experimental tests applied in the subsequent papers.

TABLE I

List of More Important Symbols

[B] = molar concentration of free, uncombined, coordinating base
E_h = electrode potential referred to the hydrogen standard
E_b = E_h at constant S_b, other conditions being specified in the text
E'_0 = E_h at 50% reduction of metalloporphyrin, $S_b = 0$, other conditions being specified in the text
e = 1 equivalent of electrons
F = the faraday
K_O = dissociation constant of oxidized base metalloporphyrin
K_R = dissociation constant of reduced base metalloporphyrin. Note, special dissociation constants are defined as needed
n = (1) number of equivalents required to reduce 1 mole of oxidant; (2) number of gm. atoms of metal per mole of oxidant
$[O_n]$ = molar concentration of oxidized metalloporphyrin containing n gm. atoms of metal ion per mole
[OOH] = molar concentration of oxidized metalloporphyrin associated with 1 mole of hydroxyl ions per mole
$[B_qO_n]$ = molar concentration of oxidized, base metalloporphyrin containing q moles of base per mole
$\epsilon' = -\log T = \epsilon cl$
p = ratio of gm. atoms of metal per mole of oxidant to gm. atoms of metal per mole of reductant
q = number of moles of base associating with 1 mole of oxidized metalloporphyrin
r = number of moles of base associating with 1 mole of reduced metalloporphyrin
$[R_m]$ = molar concentration of reduced metalloporphyrin
$[B_rR_m]$ = molar concentration of base metalloporphyrin containing r moles of base per mole
R = gas constant
$S = S_o + S_r$
S_o = sum of apparent molar concentrations of oxidized metalloporphyrin and oxidized base metalloporphyrins
S_r = sum of apparent molar concentration of reduced metalloporphyrin and reduced base metalloporphyrins
S_b = sum of apparent molar concentrations of uncombined base and base metalloporphyrins
T = absolute temperature
T = transmittance

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PART II. EXPERIMENTAL

The introduction to this series of reports (1) has indicated that we may expect to elucidate clearly the main features of systems containing metalloporphyrins and nitrogenous bases that coordinate therewith only when properties pertaining to each of the several components are varied independently. Accordingly, metal, porphyrin, and base, as well as the hydrogen ion activity and the ionic strength of the solutions, have been varied in a series of measurements of the equilibria obtaining among these components.

To initiate such studies in this laboratory metallo compounds of mesoporphyrin IX were chosen because this porphyrin is somewhat more stable than protoporphyrin. Protoporphyrin IX is the porphyrin component of those iron porphyrins previously studied by Conant and his students (2) and by Barron (3). We were surprised to find that the hydrogenation of the vinyl groups of protoporphyrin was accompanied by certain undesirable changes in the properties of the corresponding metalloporphyrins. In particular the solubilities of reduced metalloporphyrins formed from mesoporphyrin are low and this has seriously limited the range of our experiments.

Laidlaw (4), who first prepared a cobalt porphyrin, recognized that it could be oxidized and reduced. Zaleski (5) prepared manganese mesoporphyrin, which Barcroft (6) mentions can be

oxidized and reduced. It is our purpose to show that manganese and cobalt mesoporphyrins, as do metalloporphyrins which contain iron, participate in several of the equilibria previously outlined (1), more particularly in those of oxidation-reduction and association with a base.

Preparations

Hemin chloride, ferriprotoporphyrin IX chloride, was prepared from washed pig erythrocytes by a slight modification of the method of Schalfejew (7), improved by Willstätter and Fischer (8). It was thrice recrystallized by the quinine-chloroform method as suggested to us by Barron (see (9)).

Mesoporphyrin IX hydrochloride can be prepared from hemin chloride by simultaneous reduction and removal of iron. Three different methods gave indistinguishable products. The method most satisfactory in our hands was the following. Hemin chloride (3 gm.) is finely ground and suspended in formic acid (250 ml., 87 per cent). While the mixture is being heated under a reflux condenser, small portions of palladium catalyst, prepared by Adams' method (10), are added. The progress of the reaction is followed spectroscopically. There appears first the spectrum of protoporphyrin as sufficient reduction takes place to form the dissociable ferroprotoporphyrin. This spectrum gradually gives place to that of mesoporphyrin, and in order that the product may contain minimal quantities of protoporphyrin it is advisable to continue the heating until no trace of the protoporphyrin spectrum can be observed in as concentrated an ethereal solution of the reaction mixture as is compatible with distinguishing the closely placed bands of these two compounds. Heating should then be discontinued, for it is evident from what appears in the next step that additional reactions, which may be called those of "decomposition," are taking place. There will be the temptation to continue the heating to increase the yield, since considerable undissolved hemin remains. Some compromise is advisable. 2 to 3 hours are usually required. The cooled solution is filtered and poured slowly into 30 per cent ammonium acetate solution (1 liter). The precipitate is collected by centrifugation and is washed repeatedly with water. It is taken up in sodium hydroxide solution with great care to use as low a concentration of alkali

as is practicable. The solution is filtered, and the sodium "salt" of mesoporphyrin is "salted-out" with sodium tartrate. The centrifugation of this precipitate reveals the "decomposition" products, for when the material is washed with sodium tartrate solution (2 per cent), the dark, brownish colored supernatant gradually gives place to a clear solution, leaving a bright, red precipitate. It is advisable to repeat the solution in water and salting-out with sodium tartrate, and in doing so it is essential that the precipitate be "cut" sharply from the mother liquor, lest the salt in the latter prevent solution in any but large quantities of water. The sodium salt is converted into mesoporphyrin hydrochloride by throwing upon the precipitate (in the centrifuge cups) boiling 2.5 per cent hydrochloric acid solution (200 ml.). The solid dissolves instantly and the crystals of the hydrochloride form almost immediately, so that if filtration is desired before the crystallization one must operate quickly with an efficient suction filter. The crystals are washed with a little 2.5 per cent hydrochloric acid solution, and dried in air.

Identification—Samples of mesoporphyrin hydrochloride prepared by different methods were compared in the spectrophotometer. The several samples when compared in any *one* of several sorts of solution gave essentially the same absorption curves and molar extinction coefficients.

Portions of each preparation were converted to the dimethyl ester, which was recrystallized from methyl alcohol and chloroform. The "melting points" were observed in capillary tubes, immersed in a vigorously stirred bath of Crisco, electrically heated at various controlled rates. Thus it was possible to approach the expected melting point so steadily and slowly that there was ample time to make the lowest adjustments of the White potentiometer (corresponding to about 0.02°) with which a copper-constantan thermocouple was read. The thermocouple, made of tested wires, was calibrated in boiling water and in boiling naphthalene. None of the samples melted with ideal sharpness. The general picture was that described by White (11) as characteristic of a slightly impure substance. The possibility of some decomposition during the melting in open capillary tubes exposed to air cannot be excluded.

A sample of the dimethyl ester of the mesoporphyrin hydro-

chloride, prepared as described, "melted" at 215.6° and 215.7°, while a sample obtained some years ago from Professor Hans Fischer gave an average "melting point" of 213°. The several values for mesoporphyrin IX dimethyl ester, reported in the course of years from Professor Fischer's laboratory, range from 210° to as high as 216° (Fischer and Stangler (12)). Mixtures of our samples in various proportions with that from Professor Fischer "melted" at 215.1°, 215.0°, and 215.5°.

Manganese mesoporphyrin was prepared by a slight modification of the method of Zaleski (5). Mesoporphyrin hydrochloride (2.5 gm.) was dissolved in hot glacial acetic acid (75 ml.). Manganese acetate tetrahydrate, obtained from de Haën, and reported to contain not over 0.00003 per cent Fe, 0.0007 per cent heavy metals, 0.001 per cent Mg, and 0.0001 per cent Ca, was added as a solution of 5 gm. in 25 ml. of 60 per cent acetic acid. The reaction mixture was heated, with shaking to promote oxidation by air, and presently diluted with 50 ml. of water. Heating was continued until there appeared in the spectrum a band at 460 mμ characteristic of the manganese mesoporphyrin. After the spectral bands of the porphyrin had disappeared completely, the solution was concentrated *in vacuo* to 25 ml. Addition of water to the warm, concentrated solution produced a copious precipitate, which, after being washed with water and dried *in vacuo* over calcium chloride and potassium hydroxide, amounted to 1.96 gm. The material is too soluble in glacial acetic acid to be recrystallized by the quinine-chloroform method, or other methods employed with hemin chloride.

Cobalt Mesoporphyrin—If one treats a glacial acetic acid solution of mesoporphyrin hydrochloride with a solution of cobaltous acetate in the same solvent, one observes absorption bands characteristic of a metalloporphyrin, and the precipitation of fine, red, bent needles. Upon dissolving the precipitate in aqueous alkali one observes the two-banded spectrum of cobaltimesoporphyrin so that one may be misled into the belief that the original preparation is also the oxidized form. Actually it is cobaltomesoporphyrin, which is but slowly oxidized by air under the conditions of its formation, yet is rapidly oxidized in aqueous alkali. The precipitate forms so rapidly under certain circumstances that it occludes considerable mesoporphyrin, easily recognized

by the spectrum in nicotine solution. Fischer, Treibs, and Zeile (13) report similar occlusion in the preparation of protohemin. The best preparations were obtained by delaying precipitation, and operating under purified nitrogen.

Cobaltous acetate was prepared from Merck reagent $Co(NO_3)_2 \cdot 6H_2O$, "low in nickel," for which the reported limits of impurities were 0.01 per cent Ni, 0.001 per cent Fe, 0.000 per cent Cu, 0.03 per cent Zn, and 0.000 per cent Pb. The carbonate was precipitated by reagent potassium carbonate, washed free of nitrate, taken up in glacial acetic acid, and the solution concentrated *in vacuo* over potassium hydroxide and calcium chloride until the solid phase formed. 1 gm. of this material was dissolved in hot glacial acetic acid (1 liter). Mesoporphyrin hydrochloride (0.5 gm.) was dissolved in another portion of hot glacial acetic acid (0.5 liter). After each solution had been deaerated by a stream of pure nitrogen, the porphyrin solution was added dropwise to the solution of cobaltous acetate, while the latter was stirred mechanically and by the stream of nitrogen. The addition was controlled, spectroscopically, to prevent the accumulation of excess porphyrin. Precipitation began after about one-fourth of the porphyrin had been added. Finally the mixture was cooled under nitrogen, the precipitate was collected rapidly, washed in the filter with cold, oxygen-free glacial acetic acid, and dried *in vacuo* over potassium hydroxide. Yield 0.38 gm.

That the material which separates during the preparation is cobaltomesoporphyrin and that this is oxidized by air when it is brought into aqueous alkaline solution is shown quantitatively as follows: Samples were weighed into the side arms of Warburg manometer vessels, in the main chambers of which was placed aqueous alkaline solution of nicotine sufficient to dissolve the cobalt mesoporphyrin. After the vessels had attained constant temperature, the samples were allowed to dissolve and the oxygen consumption was measured in the usual manner. Proper corrections were made for the slow progressive absorption of oxygen by nicotine in alkaline solution. The results, summarized in Table I, confirm the spectroscopic evidence that the material is cobaltomesoporphyrin.

No cobalt compounds with mesoporphyrin have been found described in the literature.

Other Preparations—Mesoporphyrin dimethyl ester was combined with cobalt and with manganese by essentially the methods already described. Both the cobalt and the manganese mesoporphyrin esters have spectra similar in type and in position of the bands to the spectra of the corresponding metalloporphyrins. Either compound is insoluble in aqueous alkaline solution unless nitrogenous base be added.

Chromium metalloporphyrins should be of interest, because the well known chromium ammines resemble closely the corresponding cobalt compounds, especially in exhibiting two valence states. Every attempt to prepare chromium mesoporphyrins, by any of the reported methods for metalloporphyrin synthesis, has

TABLE I

Oxygen Absorption by Cobaltomesoporphyrin

Samples dissolved in 5 ml. of 0.2 M NaOH + 0.5 ml. of nicotine at 30°.

Weight of sample (1)	Gas absorbed (0°, 760 mm. Hg) (2)	Gas absorbed (corrected)* (3)	Oxygen absorbed by sample (4)	Oxygen equivalent to sample (5)
gm.	*c.mm.*	*c.mm.*	*moles*	*moles*
0.00932	94.4	81.55	3.6×10^{-6}	3.7×10^{-6}
0.01222	118.3	105.45	4.7×10^{-6}	4.9×10^{-6}

* A solution of 5 ml. of 0.2 M NaOH + 0.5 ml. of nicotine without cobaltomesoporphyrin absorbed oxygen steadily for 2 hours. From this experiment was obtained the correction of 12.85 c.mm. (0°, 760 mm. of Hg) subtracted from Column 2 to yield Column 3.

failed. Hill (14) and Fischer, Treibs, and Zeile (13) have also reported failure. We have found no chromium porphyrins reported in the literature.

Copper and nickel mesoporphyrins, prepared as was the cobalt compound, gave no evidence of reversible oxidation and reduction.

Analyses

Each compound was ashed, preparatory to the analysis for cobalt and for manganese, respectively, by the same procedure. Samples weighed into quartz crucibles were warmed with 50 per cent sulfuric acid on a water bath for 2 hours. The crucibles were then placed in a cold muffle furnace, and were brought gradually

to 500°. After the evolution of fumes had ceased, the temperature was brought to 800° for a short time. The crucibles were cooled and the process was repeated, the furnace being kept at 500°, at which temperature there should be no conversion of sulfate to oxide. The residues proved completely soluble.

Manganese was determined by the bismuthate method, according to Cunningham and Coltman (15). The potassium permanganate solution was standardized just before use against sodium oxalate.

TABLE II

Analyses of Metalloporphyrins

Manganese	Weight of sample	Volume $KMnO_4$	Normality $KMnO_4$	Mn found	Mn calculated	Formula
	gm.	*ml.*		*per cent*	*per cent*	
Manganimesoporphyrin	0.1482	9.81	0.1175	8.55	8.39	$C_{34}H_{36}O_4N_4MnCl$
	0.1508	10.03	0.1175	8.59	8.63	$C_{34}H_{36}O_4N_4MnOH$
	0.1511	10.14	0.1175	8.66	8.10	$C_{34}H_{36}O_4N_4MnC_2H_3O_2$

Cobalt	Weight of sample	Volume $K_3Fe(CN)_6$	Normality $K_3Fe(CN)_6$	Co found	Co calculated	Formula
	gm.	*ml.*		*per cent*	*per cent*	
Cobaltomesoporphyrin	0.1361	4.24	0.04937	9.07	9.46	$C_{34}H_{36}O_4N_4Co$
	0.1443	4.47	0.04937	9.01		
	0.1435	4.44	0.04956	9.04		
	0.1425	4.40	0.04956	9.02		

Cobalt was determined by electrometric titration of cobaltous ion in the presence of ammonia, according to Tomíček and Freiberger (16). The ferricyanide solution was standardized against sodium thiosulfate and the latter was standardized against potassium biniodate.

Table II summarizes the results of the analyses. Since the manganese compound is certainly manganimesoporphyrin, the residual positive charge of the molecule in the solid state should be matched against some negative ion. The choice of OH^- produces good agreement between the found and calculated values, and the method of formation may be in its favor. The value found

for cobalt is low for cobaltomesoporphyrin, but the evidence for this formula has been given above. The presence of some free mesoporphyrin is not excluded, although none has been detected spectroscopically.

Electrometric Measurements

Technique—The equipment and technique in general follow those of Clark (17). To preserve the basis of comparison all electrode potentials have been referred to the normal hydrogen standard after the arbitrary conventions of Clark (18).

The tendency of alkaline metalloporphyrin solutions to foam during deaeration by bubbling nitrogen led to the use of a special form of titration vessel, stirred mechanically and sealed completely from air by a mercury seal. This permitted the nitrogen to be turned off during a titration, after a preliminary deaeration.

Reduction by dithionite ($Na_2S_2O_4$), under usual conditions, proved unsatisfactory. Von Zeynek (19) has shown that sulfurous acid attacks hemin in the presence of light, and some evidence of a similar reaction appeared in our studies. Accordingly, when dithionite was used the titration vessel was carefully shielded from light. The titrations were then quite satisfactory. In the more alkaline solutions the potential of the phthiocol system[1] is sufficiently low that reduced phthiocol can be used as a reducing agent (Ball (20)). It was used in most of the reductive titrations. Satisfactory titrations were also obtained with ferricyanide as oxidizing agent following reduction with hydrogen in the presence of colloidal palladium. Barron (3) has used Paal's palladium for a similar purpose, but we preferred the catalyst made with gum arabic, according to Skita and Franck (21), in order to avoid the introduction of protein material which might form a trace of protein metalloporphyrin.

Preliminary titrations in the presence of nicotine gave potentials drifting steadily negative. This difficulty was avoided by the use of two procedures. One was distillation of the nicotine *in vacuo* under nitrogen, which yielded a water-white product; the other was the exposure of the nicotine-metalloporphyrin mixture to the air for 1 or 2 days before titration. The presence

[1] The dissertation includes a description of a modified method of synthesizing phthiocol.

of an easily oxidizable impurity is suggested. Distillation of pyridine over zinc and fractionation with a 60 cm. column failed to eliminate an impurity capable of being reduced. At the start of a titration it was necessary to wait until this material had been consumed and potentials became steady. Corrections for the amount of reducing agent so consumed are made when the titration data are analyzed by the method of Reed and Berkson (22), which was applied in every instance. The α-picoline, Eastman, was not further purified.

The pH values of the buffer-base solutions were determined by the use of the glass electrode, after the method outlined in the introductory paper (1). Since the characteristic potentials of the systems now under consideration were found independent of pH, the measurements are not as critical as for the cases studied by Davies (23) and by Vestling (24).

Potentials in Absence of Base—All attempts to titrate cobalt and manganese mesoporphyrins in the absence of coordinating base failed to give reliable data. The reductants are so slightly soluble in aqueous alkaline solution that precipitation began after a slight portion of reducing agent had been added to a solution in which the metalloporphyrin was 0.000025 M, and the electrode potential then continued to drift as if reductant were being continuously removed. This explanation seems adequate in the present instance but that another factor may be involved is suggested by the experience of Davies and Vestling with other metalloporphyrins.

Potentials in Presence of Base—When any suitable base is added to the cobalt or manganese metalloporphyrin system, the electrode potentials become remarkably steady, and tests can be made of some, if not all, of the relationships discussed in Paper I of this series (1). Results are reported with nicotine, pyridine, and α-picoline.

Titrations in the presence of piperidine yielded potentials drifting negative so rapidly that measurements were precluded.

Barron (3) and Davies (23) have shown that cyanide iron porphyrin systems may easily be titrated. Attempts to titrate cyanide cobalt- and manganese mesoporphyrins met with peculiar difficulties. No steady potentials were obtained except in the region poised by the phthiocol system, used as reducing agent.

No reduction appeared to be brought about by colloidal palladium and hydrogen. Barron (3) also reports this for iron protoporphyrin and cyanide. When solid cobaltomesoporphyrin was added to deaerated buffer solution containing cyanide ion, the metalloporphyrin did not dissolve. Since there will appear some spectroscopic evidence that cyanide ion coordinates with cobaltimesoporphyrin, it may be suggested that it coordinates with this

TABLE III

α-Picoline Manganese Mesoporphyrin System

Titration of α-picoline manganimesoporphyrin with $Na_2S_2O_4$ in the dark at constant pH and constant α-picoline concentration. Demonstration that $n = 1$. α-Picoline = 0.93 M; total pigment = 2.5×10^{-4} M; $Na_2S_2O_4$ = *ca.* 1.36×10^{-3} N; pH of phosphate buffer + α-picoline = 7.60; $\mu = 0.22$; temperature = 30°; $d = 0.66$ ml.; 100 per cent reduction at $y = 7.99$ ml.

y	$y - d$	Reduction	$0.06011 \times \log \frac{S_o}{S_r}$	E_h	E_b	Deviation from −0.2958
ml.	*ml.*	*per cent*		*volt*	*volt*	*volt*
1.50	0.84	11.46	+0.0534	−0.2452	(−0.2986)	−0.0028
2.00	1.34	18.28	+0.0391	−0.2573	−0.2964	−0.0006
2.50	1.84	25.10	+0.0285	−0.2674	−0.2959	−0.0001
3.00	2.34	31.92	+0.0198	−0.2760	−0.2958	0.0000
3.50	2.84	38.74	+0.0120	−0.2838	−0.2958	0.0000
4.00	3.34	45.57	+0.0046	−0.2913	−0.2959	−0.0001
4.50	3.84	52.38	−0.0025	−0.2985	−0.2960	−0.0002
5.00	4.34	59.21	−0.0097	−0.3055	−0.2958	0.0000
5.50	4.84	66.03	−0.0174	−0.3133	−0.2959	−0.0001
6.00	5.34	72.85	−0.0258	−0.3216	−0.2958	0.0000
6.50	5.84	79.67	−0.0357	−0.3308	−0.2951	+0.0007
7.00	6.34	86.49	−0.0485	−0.3427	(−0.2942)	+0.0016
7.60	6.94	94.68	−0.0752	−0.3641	(−0.2889)	+0.0069
Average					−0.2958	

form only. If the reductant does not coordinate, its insolubility will make potentiometric titrations impossible.

Descriptions of Relations.—Paper I (1) outlined various propositions, some of which will be submitted now to experimental tests. For the sake of brevity we shall refer the reader to Paper I for definitions of the symbols and propositions now used.

Determination of p *and* n, *by Propositions I and II*.—Tables

III and IV so well represent the results of numerous titrations as to make unnecessary the publication of the detail in all cases.

Rectification of the titration curves by the method of Reed and Berkson (22) according to Proposition XVI indicated clearly that p = 1 and $n = 1$, subject only to the slight and, we think, negligible doubt noted in Paper I.

TABLE IV

Nicotine Cobalt Mesoporphyrin System

Titration of nicotine cobaltimesoporphyrin with reduced phthiocol at constant pH and constant nicotine concentration. Demonstration that $n = 1$. Nicotine = 0.30 M; total pigment = 2.5×10^{-4} M; phthiocol = *ca.* 6.4×10^{-4} M; pH of phosphate buffer + nicotine = *ca.* 11.4; $\mu = 0.45$; temperature = 30°; $d = 0.08$ ml.; 100 per cent reduction at $y = 7.92$ ml.

y	$y - d$	Reduction	$0.06011 \times \log \frac{S_o}{S_r}$	E_h	E_b	Deviation from −0.1992
ml.	*ml.*	*per cent*		*volt*	*volt*	*volt*
0.50	0.42	5.36	+0.0750	−0.1294	(−0.2044)	−0.0052
1.00	0.92	11.73	+0.0527	−0.1473	(−0.2000)	−0.0008
1.50	1.42	18.11	+0.0394	−0.1599	−0.1993	−0.0001
2.00	1.92	24.49	+0.0294	−0.1696	−0.1990	+0.0002
2.50	2.42	30.87	+0.0211	−0.1782	−0.1993	−0.0001
3.00	2.92	37.25	+0.0136	−0.1855	−0.1991	+0.0001
3.50	3.42	43.62	+0.0067	−0.1924	−0.1991	+0.0001
4.00	3.92	50.00	0.0000	−0.1992	−0.1992	0.0000
4.50	4.42	56.38	−0.0067	−0.2059	−0.1992	0.0000
5.00	4.92	62.76	−0.0136	−0.2128	−0.1992	0.0000
5.50	5.42	69.13	−0.0211	−0.2203	−0.1992	0.0000
6.00	5.92	75.51	−0.0294	−0.2286	−0.1992	0.0000
6.50	6.42	81.89	−0.0394	−0.2389	−0.1995	−0.0003
7.00	6.92	88.27	−0.0527	−0.2522	−0.1995	−0.0003
7.50	7.42	94.64	−0.0750	−0.2762	(−0.2012)	−0.0020
Average					−0.1992	

The values of d were always small and varied with the base used. With pyridine and α-picoline d was always positive, but with nicotine sometimes negative, indicating reduction of some metalloporphyrin by the previously mentioned, reducing impurity.

The theory demands that the concentration of free base, [B], the pH value, and the ionic strength of the solution, as well as

the temperature, must remain constant if the constants p and n are to be evaluated. Estimates indicate that, when S_b remains constant, the change in [B] due to the difference in dissociation constants of the oxidized and reduced base metalloporphyrins is negligible. It will presently be shown that there should be no correction for pH changes during the titration of these systems, and only a slight correction, which may be neglected, for change in ionic strength during titration. The agreement among the values of E_b, when calculated by means of the coefficient 0.06011 for a 1 equivalent change, is, therefore, excellent evidence that $p = 1$ and $n = 1$ for all the systems reported here.

There remains the possibility that the metalloporphyrin systems in the absence of base have values of p and n greater than 1.

Effect of pH—The several individual titration curves, each analyzed by Proposition XVI, provide a series of values of E_b at different values of pH. The impracticability of making direct determinations of E'_0 make it unsafe to do more than reduce the values to a common value of S_b which has been taken to be 0.9 M, since this covers the cases. Corrections for variations in the ionic strength of the various buffer solutions have been made by means of an empirical equation that will be described later.

The assembled results are shown in Fig. 1 and clearly indicate that $\Delta E_h/\Delta \text{ pH} = 0$. While there appear slight discrepancies, we attribute them partly to experimental error and partly to the difficulty of reducing the data to a common basis. According to a corollary of Proposition X the relation demonstrated shows that within the range of pH studied there is no difference between oxidized and reduced base metalloporphyrins with respect to association with H^+ or OH^-. It does not show that both may not be associated with OH^-, for example.

Reduction of the data to a common basis of S_b does not provide a strictly logical basis of comparison for obvious reasons. None the less the relations made evident in Fig. 1 retain sufficient comparative value to be interesting.

The E'_0/pH curve of the phthiocol system (Curve B of Fig. 1), which is more negative than the metalloporphyrin systems in the alkaline range, crosses the curves for these systems at lower values of pH. For example, if a solution of manganimesoporphyrin and pyridine be reduced by phthiocol at pH 12, the acidifica-

tion of the solution to pH 7.5 should be more than sufficient to reverse the direction of the reaction. This has been confirmed experimentally for the cobalt and manganese metalloporphyrins in the presence of pyridine. This is another of the several examples exhibiting concretely the integration of acid-base and oxidation-reduction equilibria in a continuum.

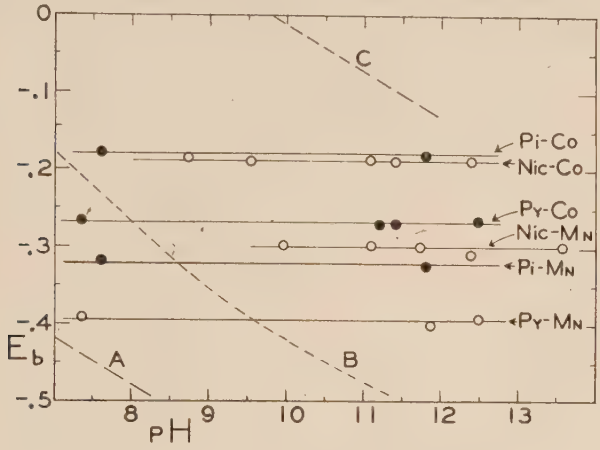

FIG. 1. Relation of potential E_b, at constant S_b, to pH. All data are reduced to $\mu = 0$, $S_b = 0.9$, $S = 2.5 \times 10^{-4}$ M, $S_o = S_r$, 30°. *Pi-Co*, α-picoline + cobalt mesoporphyrin; *Nic-Co*, nicotine + cobalt mesoporphyrin; *Py-Co*, pyridine + cobalt mesoporphyrin; *Nic-Mn*, nicotine + manganese mesoporphyrin; *Pi-Mn*, α-picoline + manganese mesoporphyrin; *Py-Mn*, pyridine + manganese mesoporphyrin. Curve A, hydrogen at 1 atmosphere; Curve B, phthiocol; Curve C, pyridine + iron protoporphyrin (heme).

Association Experiments—The extraordinary limitations of low solubility in the absence of base, already mentioned, and of incomplete association in the presence of enormous concentration of base, now to be described, prevented determination of the association constants by the method of establishing the limiting potentials and the complete association curves. The pyridine manganese mesoporphyrin system alone yielded data extensive enough to be of value in checking the laws of association. Even in this instance it was necessary to operate with the system only slightly reduced in order to obviate the difficulties arising from the precipi-

tation of manganomesoporphyrin at low pyridine concentrations. The less extensive data of other systems revealed nothing at variance with the conclusions which we shall draw, and their detailed consideration is omitted.

Fig. 2 summarizes the data. They are plotted in such a way as to facilitate the graphical analysis described in Paper I as Proposition V. The smooth curve of Fig. 2, constructed with the aid of the graphical analysis and the simplest assumptions, is theo-

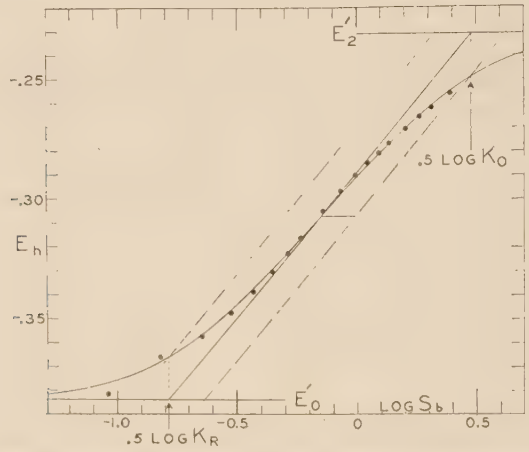

FIG. 2. Effect of adding pyridine to the manganese mesoporphyrin system, 5 per cent reduced. Initial total pigment, $[S]$, $= 2.5 \times 10^{-4}$ M; pH $= 12.35$; $\mu = 0.1$; temperature $= 30°$. The curve is drawn with $q = r = 2$, $K_O = 9.26$, $K_R = 2.64 \times 10^{-2}$, $E'_0 = -0.384$ volt, $E_2 = -0.231$ volt. E'_0 (at 50 per cent reduction and $\mu = 0$) $= -0.4830$ volt.

retical for the case in which $q = r = 2$ and the constants have the numerical values indicated in the legend.

Irrespective of the guidance that the theoretical curve may seem to impose, the following conclusions may be drawn.

The potential increases with increase of pyridine. This indicates that K_O is greater than K_R, according to Proposition III. The maximum of the slope, dE_h/d (log S_b), is very close to the value 0.1202. Could it be proved with certainty that the value of K_O/K_R were large enough to make Proposition VI strictly applicable, this would indicate that r has the value 2 ($0.0601r = 0.1202$). That K_O/K_R is sufficiently large is suggested by the fact that the

slope of the tangent does approach the indicated, integral multiple of 0.0601 and also by the spread between the points of maximal curvature.

Since the experimental curve departs from the maximal slope as S_b increases, q must be greater than 0 according to Proposition VIII.

Except for the possibility of an extraordinary distribution of the values of the dissociation constants, it remains fairly certain that $r = 2$. If K_R be in fact the product $K_{R_1}K_{R_2}$, the values of the individual constants are not very different.

The outstanding uncertainty is the conduct of the system at high concentration of pyridine and this concerns the coordination with manganimesoporphyrin. Obviously the data are not extensive enough for testing Proposition IV or IX. They are also insufficient for the adequate application of the rectification test of Proposition XV. None the less, neither the assumption (*a*) $q = 1$, nor the assumption (*b*) $q = 2$, and no stepwise association, leads Proposition XV to a satisfactory result and this suggests stepwise association. Of this all that can be said is the following. Once the general form of the association curve has been satisfactorily defined with respect to first order approximation as it has been by the curve of Fig. 2, it becomes possible to reproduce this curve rather closely by preserving the same product $K_O = K_{O_1}K_{O_2}$ with various distributions of the individual values of K_{O_1} and K_{O_2}.

This shows clearly the limitations of the potentiometric method when the properties of a system restrict the extent of exploration. Particular attention may be called to this, since other limitations are inherent in the interpretation of spectrophotometric data and conclusions will have to be pieced together from the results of both methods.

Effect of Ionic Strength of Solution on Potential Pending the development of the chief features of the metalloporphyrin systems it was thought advisable to give but secondary attention to the effect of variations in the ionic strength. Such variations do affect the oxidation-reduction potential. Significant results may attend a more exhaustive investigation than the following orienting experiment.

With the equations given in Paper I it may be shown that

when $p = m = n = 1$, $r = q = 2$, and $S_o S_r$ = constant, the potential should vary with activity coefficients as follows: When [B] = 0

$$E_h \propto \frac{RT}{F} \ln \frac{\gamma_O}{\gamma_R} \tag{1}$$

When [B] $\gg K_O$ and K_R

$$E_h \propto \frac{RT}{F} \ln \frac{\gamma_{OB_2}}{\gamma_{RB_2}} \tag{2}$$

Between these extremes E_h is a complicated function of the four activity coefficients mentioned and of $\gamma_B{}^2$. If z_r is the valence of the reductant, and z_o the valence of the oxidant, the limiting law of Debye and Hückel would give, at 30°,

$$E_h \propto 0.03006\ (z_r{}^2 - z_o{}^2)\ \sqrt{\mu} \tag{3}$$

for either Equation 1 or 2.

The absence of a pH effect in the systems under discussion suggests, but does not prove, that there is no change of valence when a neutral substance (like pyridine or nicotine in alkaline solution) coordinates with the metalloporphyrins. Therefore we may proceed tentatively with Equation 3.

There are two carboxyl groups in mesoporphyrin. Let it be assumed that these two groups as they occur in the metalloporphyrin are completely ionized and function only as ionized groups at high pH values. If either of the ions Mn^{++} or Mn^{+++} replaces 2 protons at the coordination center, the net charge of manganomesoporphyrin should be two (negative kind) and of manganimesoporphyrin should be one (negative kind). Let the same charges be assumed for the nicotine complexes. Accordingly, and if all conditions be constant except the ionic strength of the solution, the slope of the limiting Debye-Hückel relation should be

$$\frac{dE_h}{d\sqrt{\mu}} = 0.0902 \tag{4}$$

To a solution of manganese mesoporphyrin about 23 per cent reduced with phthiocol, 0.005 M with respect to sodium hydroxide, and 0.3 M with respect to nicotine, there were added successive

portions of a solution of potassium chloride, containing the same concentration of nicotine as did the original solution after the addition of the phthiocol. Fig. 3 summarizes the experimental data.

The dotted line of Fig. 3 is drawn with slope $\Delta E_h/\Delta\sqrt{\mu} = 0.0902$ corresponding to $z_r = 2$ and $z_o = 1$. The curve through the experimental points was drawn with the extended equation

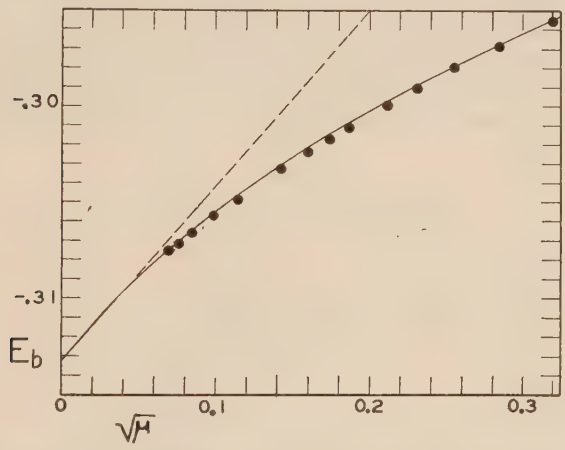

FIG. 3. Relation of potential to ionic strength in the nicotine manganese mesoporphyrin system. The system was about 23 per cent reduced with phthiocol. Total pigment = 2.76×10^{-4} M; nicotine = 0.30 M; temperature = 30°; straight line $\Delta E/\Delta\sqrt{\mu} = 0.09$.

of Debye and Hückel and the arbitrary value 6×10^{-8} cm. for a, the "ionic diameter." The complete equation is

$$E_h \propto \frac{0.0902\sqrt{\mu}}{1 + 1.98\sqrt{\mu}} \qquad (5)$$

To use the apparent approach to the predicted limiting slope as an argument in favor of the specified ionic charges it will have to be shown that the natures of the molecules under consideration do not seriously invalidate the applicability of the limiting law of Debye and Hückel.

Log Transmittance Curves—The curves of various cobalt mesoporphyrin systems are presented in Figs. 4, 5, 7, and 8.

Since spectrophotometric characterization of porphyrin derivatives is valuable in furnishing constants of reference, Table V

records the molar extinction coefficients, ϵ, at specific wavelengths, for the cobalti- and manganimesoporphyrins. These constants are calculated according to the equation

$$-\log T = \epsilon cl$$

where T is transmittance, c is molar concentration, and l is cell length in cm.

It is worth remarking, with respect to these curves, that of the bases tested only cyanide produces a striking change in the location of the absorption bands. This is in contrast to the behavior of the iron porphyrin systems. Pauling and Coryell

TABLE V

Molar Extinction Coefficients

Concentration of pigment × 10^5	λ	−Log T	ϵ
Manganimesoporphyrin. NaOH = 0.01 M; cell length = 4 cm.; slit width = 0.1 mm.			
	$m\mu$		
4.95	552	1.2676	6.4×10^3
0.99	552	0.3372	8.5×10^3
Cobaltimesoporphyrin. NaOH = 0.005 M; cell length = 4 cm.; slit width = 0.1 mm.			
2.535	545	0.7894	7.8×10^3
2.415	545	0.7293	7.6×10^3
2.488	545	0.7359	7.4×10^3
2.488	560	1.2017	12.1×10^3
2.488	525	1.1654	11.7×10^3

(25) have pointed out that there is a correlation between the type of spectrum and the type of metal-porphyrin bond, as revealed by magnetic measurements, in ferri- and ferroprotoporphyrin, in a number of their base complexes, and in nickel protoporphyrin. This would suggest that in cobaltimesoporphyrin, and perhaps in other systems observed, there is no change in metal-porphyrin bond type upon the addition of nicotine, pyridine, or α-picoline.

Viewing the log transmittance curves upside down in order that we may speak of absorption "peaks" one notices that cobaltomesoporphyrin, nicotine cobaltomesoporphyrin, and cyanide

cobaltomesoporphyrin each has the sign of a "foot-hill" beside the "peak" and at lower wave-length. On the other hand, in the case of the manganomesoporphyrin (Fig. 10; see "Supplementary note") a similar "foot-hill" is at higher wave-length.

The curves for cobaltimesoporphyrin in the presence and absence of nicotine, shown in Fig. 4, coincide so nearly that they cannot be used in the study of the association equilibrium ac-

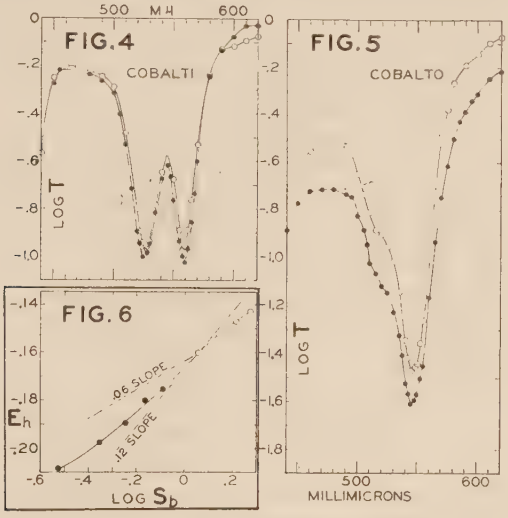

FIG. 4. Log transmittance curves. ○ represents nicotine cobaltimesoporphyrin IX. $S = 8.96 \times 10^{-6}$ M; $S_b = 0.25$ M; NaOH = 0.02 M; 10 cm. tube. ● same without nicotine.

FIG. 5. Log transmittance curves. ○ represents nicotine cobaltomesoporphyrin IX. $S = 8.96 \times 10^{-6}$ M; $S_b = 0.25$ M; NaOH = 0.02 M; 10 cm. tube. ● same without nicotine.

FIG. 6. Relation of E_h to log S_b at constant degree of reduction. ● represents cobalt mesoporphyrin + nicotine; ○ cobalt mesoporphyrin + pyridine.

cording to the method outlined in Paper I (1). The same is true for the pyridine and α-picoline systems, whose curves are essentially identical with the curves in Fig. 4. The curve for cobaltomesoporphyrin (Fig. 5) may not be strictly comparable with that for cobaltomesoporphyrin plus nicotine, since the concentration is near the solubility limit of the free cobaltomesoporphyrin, and a solid phase may have separated in particles large

enough to alter the transmittance. Assuming that the observed differences are not artifacts, they remain too small to be used in the study of the association with the instrument available.

The addition of cyanide to cobaltimesoporphyrin does produce a marked alteration in the curve, as shown in Fig. 7. The differences should be capable of use in studying the course of association to form a cyanide cobaltimesoporphyrin, yet we have not succeeded in analyzing the data according to any equilibrium equa-

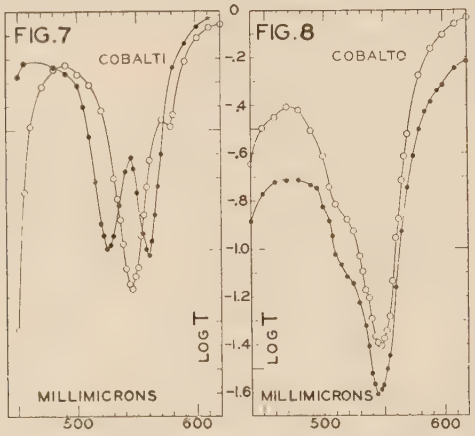

FIG. 7. Log transmittance curves. ○ represents cyanide cobaltimesoporphyrin. $S = 8.96 \times 10^{-6}$ M; $S_b = 0.008$ M; NaOH = 0.02 M; 10 cm. tube. ● same without cyanide.

FIG. 8. Log transmittance curves. ○ represents cyanide cobaltomesoporphyrin. $S = 8.96 \times 10^{-6}$ M; $S_b = 0.008$ M; NaOH = 0.02 M; 10 cm. tube. ● same without cyanide.

tion. Fig. 8 shows that the curves for the two reduced systems are similar.

Comparison of Figs. 4 to 6 is of special interest. The formation of a nicotine cobaltomesoporphyrin is indicated by the change of potential (*cf.* Fig. 6). Fig. 6 also shows evidence of a combination between cobaltomesoporphyrin and pyridine. On the other hand Fig. 4 suggests no combination of the cobalti compound with nicotine and less quantitative studies suggest little distinct spectral evidence of the combination with pyridine. Now potentiometric data could render a decision on this elementary question were

the properties of the system such as to permit extensive potentiometric measurements and this they do not permit. We hope that we are not overlooking something of significance when, for the sake of simplicity and on the basis of instability of potentials and formation of precipitates, we reject from Fig. 6 abnormally high potentials observed at the lower concentrations of pyridine. The residual and more reliable data which alone are shown suggest a definite departure from the "0.12 slope" and this can be taken as presumptive evidence that $q > 0$ (see Proposition VIII, but note the underlying assumptions).

SUMMARY

Manganese mesoporphyrin and cobalt mesoporphyrin have been prepared and characterized. The latter is believed to be a new compound.

Potentiometric titration curves of systems containing each one of these compounds and pyridine, or nicotine, or α-picoline, have been analyzed by an objective method and have yielded the conclusions that there is no evidence of polymerization and that 1 equivalent is concerned in the oxidation-reduction process. Numerical values of the characterizing potentials are recorded.

All other conditions being the same within narrow experimental limitations, dE_h/d pH $= 0$ in all the systems.

Limitations imposed by the properties of one or another of the components have prevented the determination of the full course of the association between these metalloporphyrins and any one of the bases mentioned; but sufficient of the course of association between pyridine and mixed mangani- and manganomesoporphyrin has been followed to draw the following conclusions. It is certain that pyridine associates better with manganomesoporphyrin than with manganimesoporphyrin. It is reasonably certain that 2 molecules of pyridine associate with 1 of manganomesoporphyrin and that at least 1 pyridine associates with manganimesoporphyrin. It is not improbable that 2 pyridines associate with manganimesoporphyrin. The best agreements so far found between the data and a theoretical formulation are summarized by the following numerical values of constants defined in Paper I: $p = m = n = 1$, $q = r = 2$, $K_O = 9.26$, $K_R = 0.0264$, $E'_0 = -0.4830$ volt, $E_2 = -0.3300$ volt. The way is opened

for a formulation in terms of stepwise association between pyridine and manganimesoporphyrin.

The simplified equation of Debye and Hückel, relating activity coefficients to ionic strength, has been extended to describe the effect of changing ionic strength on the potential of the nicotine manganese mesoporphyrin system. While the results of the experimental test are not decisive, they suggest that, if factors not now considered can be shown not to invalidate the treatment, the net charge of nicotine manganimesoporphyrin is one and that of the mangano compound is two.

Spectral data for several of the systems are recorded.

SUPPLEMENTARY NOTE

By W. MANSFIELD CLARK

Taylor's potentiometric data could not decide the conduct of manganimesoporphyrin with pyridine and the spectrophotometric equipment with which he attempted to solve this and similar questions did not meet the requirements of precision that the problem demanded. Therefore he generously consented to withhold publication of his dissertation pending my attempt to meet the situation by constructing a photoelectric spectrophotometer.

The instrument used is described in Paper V.

Figs. 9 and 10 show log transmittance curves of the systems concerned. Fig. 11 records one of several sets of data for the association of manganimesoporphyrin and pyridine and one of several sets of data for the association with manganomesoporphyrin. None of these curves yielded satisfactory results by the estimates of Proposition XII or by the rectification method of Proposition XIV and therefore interpretations of the curves require the exercise of judgment.

Even if the mangano system follows a simple law, it will be noted that the maximal difference, $\epsilon'_2 - \epsilon'_1$, is so small as to require an extreme precision of measurement to satisfy any requirement exactly. Indeed, it is surprising that the data align as well as they do. What is of first order importance is that the mid-point, estimated to occur at $\log S_b = -0.68$, is not seriously inconsistent with Taylor's first order approximation of K_R. His value, estimated on the basis of $r = 2$, would predict that the half transformation point should occur where shown by the center of the

dotted curve of Fig. 11. Corrections for the approximation $S_b \doteqdot [B]$ are not significant. Were it true that K_R should be the product of K_{R_1} and K_{R_2} and that the values of the latter differ slightly, there is opened the possibility of explaining qualitatively the departure of the spectral curve from the theoretical curve for $r = 2$. Such a gratuitous, *ad hoc* assumption is mentioned only because it and the limitations of a theoretical treatment to

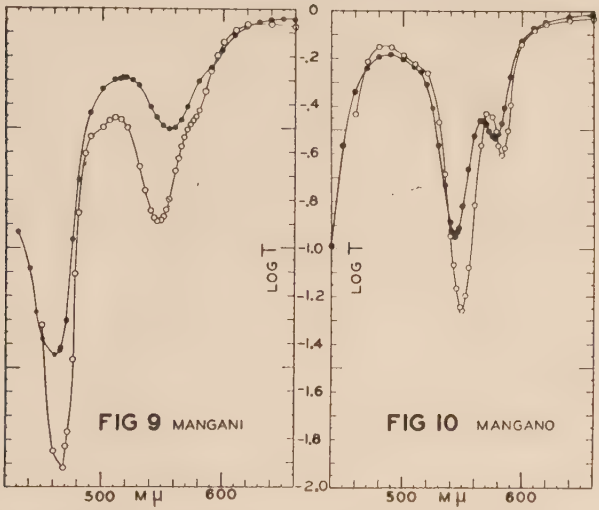

FIG. 9. Log transmittance curves for manganese mesoporphyrin IX. Approximately 0.053 gm. + 0.025 M KOH per liter in a 1 cm. tube was used. ● represents manganimesoporphyrin; ○ pyridine manganimesoporphyrin (1.24 M pyridine).

FIG. 10. Log transmittance curves for manganese mesoporphyrin IX. Approximately 0.053 gm. + 0.025 M KOH per liter in a 1 cm. tube was used. ● represents manganomesoporphyrin; ○ pyridine manganomesoporphyrin (1.24 M pyridine).

two absorbing species do place the spectral data under suspicion except with regard to the order of magnitude of K_R. In this they confirm Taylor's potentiometric estimate as well as could be expected from the application of Proposition XIII.

Use of Propositions XII and XIV in analyzing the spectral data of the mangani system (Fig. 11) yielded unsatisfactory results the best of which signifies that $q = 3$ on the basis of no

stepwise association. The curve drawn on this basis is the dotted line of Fig. 11. Unless the whole picture, as it has been pieced together up to this point, be reconstructed, this value of q is impossible, since it and the attendant estimates of K_R and K_O would lead the potentiometric curve over a "hill" to declining values of E_h with increase of S_b. Such was not found and the assumption must be rejected. Furthermore, interpretations of the spectral data are put under suspicion by the observation that Beer's law

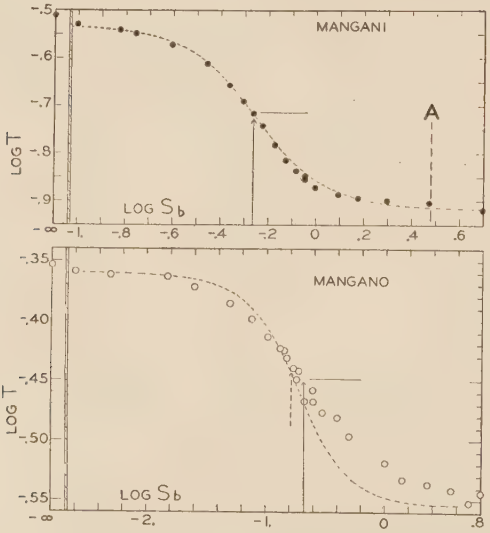

FIG. 11. Relation of log transmittance to log [pyridine]. S_b = molar concentration of pyridine. Manganimesoporphyrin, approximately 0.053 gm. + 0.05 M KOH per liter in a 1 cm. tube, mμ 548; manganomesoporphyrin IX, approximately 0.022 gm. + 0.01 M KOH per liter in a 1 cm. tube, mμ 550.

was not followed strictly by solutions of manganimesoporphyrin (see Table VI). This may be an evidence that there is change in state of aggregation of the manganimesoporphyrin. Evidence suggesting this has escaped notice previously for reasons already noted.

Examination of the consequences of the apparent failure of Beer's law in the hope of correcting the raw data preparatory to rectification by Proposition XIV was judged to be a case of "strain-

ing at a gnat and swallowing a camel" when the implications of the following remarkable fact were seen.

The order of magnitude of log S_b at which the spectral data indicate half transformation of manganimesoporphyrin to a pyridine complex is entirely inconsistent with Taylor's first, tentative estimate of the value of K_o made on the basis that $q = 2$. That estimate would demand the curve of Fig. 11 to be displaced far to the right with the center at log $S_b = +0.48$ (Point A). Conversely, use of the spectrophotometrically estimated constant would throw

TABLE VI

Effect of Dilution on Transmittance of Manganimesoporphyrin

Solution A, approximately 5×10^{-4} M manganimesoporphyrin in 0.02 M KOH; diluted with 0.02 M KOH; cell length=1 cm. Assume $-\log T = \epsilon C$.

Weight, Solution A per 100 ml.	$-\log T$	$\left(\frac{-\log T}{\text{Weight}}\right)$	Approximate concentration $\times 10^6$	Log concentration	ϵ
gm.					
0.4231	0.0144	0.0340	2.115	−5.675	6809
1.229	0.0411	0.0334	6.145	−5.211	6688
2.441	0.0806	0.0330	12.205	−4.914	6604
4.885	0.1553	0.0318	24.43	−4.613	6357
9.765	0.2983*	0.0305	48.83	−4.311	6109
14.599	0.4396*	0.0301	73.00	−4.137	6022

* Tyndall beam distinct.

a theoretical association curve quite off the track followed by Taylor's potentiometric data.

Taylor has opened the question of whether or not there is a stepwise association and has noted that if such be the case the theoretical curve of his Fig. 2 can be very nearly reproduced by preserving his value of K_o but redefining K_o as the product $K_{o_1}K_{o_2}$, to the separate constants of which are assigned empirically various values.

Now the analysis of spectral data according to the methods outlined in Paper I (1) depend upon the assumption that there are only two absorbing species. Stepwise association calls for more than two absorbing species.

But let us make the following assumptions *ad hoc*: (*a*) that absorption by the species OB_2 is only slightly greater than ab-

sorption by the species OB; (*b*) that K_{O_1} is considerably greater than K_{O_2}.

The result would be a distortion of the spectral curve but the center of this curve would be approximately where $\log K_{O_2} = \log S_b$. On these assumptions K_{O_2} is estimated to be 0.54. Let this be applied to the potentiometric curve. Taylor's estimate of K_O is 9.26. If this be $K_{O_1}K_{O_2}$ and K_{O_2} be 0.54, $K_{O_1} = 17.15$. With these values and Taylor's $K_R = 0.0264$ his curve (Fig. 2) is so nearly reproduced that only slight empirical readjustments of the constants would be required to make the new theoretical curve fit the data as well as the old. The specifications for this first trial are

$$E_h = E'_0 + 0.0601 \log \frac{K_{O_1}K_{O_2}}{K_R} + 0.0601 \log \frac{K_R + S_b^2}{K_{O_1}K_{O_2} + K_{O_2}S_b + S_b^2}$$

where $E'_0 = -0.3869$ (uncorrected to standard conditions), $K_R = 0.0264$ (Taylor), $K_{O_1} = 17.15$, $K_{O_2} = 0.54$ (spectral estimate), $K_{O_1}K_{O_2} = 9.26$ (Taylor).

Furthermore, the adoption of the spectral value but with the assumption that $q = 1$ will not give a potentiometric curve fitting Taylor's data.

Although this hypothesis roughly establishes consistency between the potentiometric and spectrophotometric data, it is not certain that it is the only way to do so. On the other hand the case illustrates admirably how misleading might be spectrophotometric data when considered alone.

Obviously the properties of these systems are poorly adapted to the experimental elucidation of some features of association between metalloporphyrins and bases and therefore we shall do well to turn to other systems for developments.

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Vita

John Fuller Taylor, the author of this dissertation, was born in Jamestown, New York, on June 10, 1912. He attended the public schools in Jamestown, in Bemus Point, and in Barneveld, New York, and graduated from the Holland Patent High School in 1929.

He then entered Cornell University, made Chemistry his major subject, and graduated as a Bachelor of Arts in the class of 1933. At this time he was awarded the Francis P. Garvan Fellowship in Chemistry for New York State at The Johns Hopkins University.

He entered the School of Higher Studies, The Johns Hopkins University, in September, 1933, and after one year in the Department of Chemistry he transferred to the Department of Physiological Chemistry, in the School of Medicine. There he carried out the research prerequisite of the degree of Doctor of Philosophy.